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Contribution of the Quartermaster Research and Development Laboratories, Philadelphia, Pennsylvania; the Osborn Botanical Laboratories, Yale University, New Haven, Connecticut; and the Biological Laboratories, Harvard University, Cambridge, Massachusetts

Abstract

Data have been presented relating to the growth of 11 species of microorganisms isolated from deteriorated cotton fabrics on various derivatives of glucose, mannose, cellobiose, and cellulose. A high degree of specificity was exhibited. As long as there was at least one firmly bound substituent in every anhydroglucose unit, the resulting derivative was not susceptible to microbiological attack. Under such conditions the nature of the substituent has relatively little influence on the degree of resistance imparted. This was considered to be promising theoretical support for the underlying premise on which the idea of multipurpose topochemical reactions as a means of mildewproofing cotton fabrics was proposed.

SINCE DORÉE'S DISCOVERY in 1920 [2] of the resistance of cellulose acetate to sea water much work has been done on the topochemical acetylation of cellulosic fibers as a means of mildewproofing. Thaysen and associates [8, 9] in England were among the pioneers in this field. Investigations were continued in this country, chiefly at the Southern Regional Research Laboratory [3]. During recent years methods of imparting mildew-resistance by topochemical reactions have been expanded [7] into

TABLE I. GROWTH OF FUNGI ON SUGAR DERIVATIVES

Sugar derivatives*	*Aspergillus niger* PQD 72A	*Aspergillus flavipes* Fla. A14	*Pestalotia* sp. PQD 79C	*Chaetomium globosum* USDA 1042.4	*Gliocladium* sp. PQD 3A	*Penicillium* sp. USDA 15.1	*Memmoniella echinata* PQD 1C	*Gliomastix convoluta* PQD 4C	*Myrothecium verrucaria* USDA 1334.2
Glucose	+	+	+	+	+	+	+	+	+
3-Methyl-α-*D*-glucose	?	−	−	−	−	?	−	?	−
2,3,6-Triethyl-*D*-glucose	−	−	−	−	−	?	−	−	−
Tetramethylglucopyranose	−	−	−	−	−	−	−	−	−
Pentacetyl-α-*D*-glucose	−	−	?	−	−	−	−	−	−
Pentacetyl-β-*D*-glucose	−	−	?	−	−	−	−	−	−
Glucose phenylosazone	−	−	−	−	−	−	−	−	−
Methyl-α-*D*-glucoside	?	−	−	?	?	+	−	+	−
Methyl-β-*D*-glucoside	+	+	+	+	+	+	+	+	+
Methyl-2,3,6-triethyl-β-*D*-glucoside	−	−	−	−	−	−	−	−	−
Mannose	+	+	+	+	+	+	+	+	+
4-Methyl-*D*-mannose	−	−	−	−	−	?	−	−	−
Methyl-α-*D*-mannoside	−	−	−	?	−	?	−	?	−
Methyl-4-methyl-α-*D*-mannoside	−	−	−	?	−	?	−	−	−
Cellobiose	+	+	+	+	+	+	+	+	+
Cellobiose heptacetate	−	−	−	−	−	−	−	−	−
Cellobiose octacetate	−	−	−	−	−	−	−	−	−
Heptacetyl ethylcellobioside	−	−	−	−	−	−	−	−	−

* Appreciation is expressed to Drs. C. S. Hudson, M. L. Wolfrom, and R. S. Bower for these compounds.

a general approach to the problem of simultaneously, and by a single chemical reaction, rendering cellulose mildew-resistant and flameproof, repellent to water, and/or resistant to light. These attempts have been based on the premise that within broad limits the nature of the chemical substituent does not itself fundamentally determine the degree of mildew-resistance. Such resistance *per se* is imparted to the cellulose molecule by chemical substituents bound to the molecule in the proper position and numbers. Accordingly, it seems possible to select substituents which, because of their inherent nature, impart other desirable properties to the fabric. The present paper presents data in support of these generalizations. It also attempts to elucidate further the mechanism of breakdown of cellulose molecules by microorganisms.

TABLE II. GROWTH OF *Myrothecium verrucaria* ON CELLULOSE DERIVATIVES

Preparation	Source*	Description	% weight loss†	Growth rating
Filter paper	Whatman	Ground to 60 mesh	69	+++
Oxidized cellulose	E. K.	Prepared with nitrogen oxide	11	+
Methyl cellulose	Dow	D.S. > 1.0	8	0—+
Methyl cellulose	Dow	D.S. = 2.0	8	+
Ethyl cellulose	Dow	D.S. = 2.25 to 2.58	0	0
Cellulose acetate	S. R. R. L.	D.S. = 1.0; 22.3% AcO	0	0
Cellulose triacetate	E. K.	—	0	0
Cellulose acetate butyrate	E. K.	16% butyryl	0	0
Cellulose acetate hydrogen phthalate	E. K.	—	8	0
Cellulose acetate stearate	E. K.	—	1	0—+
Tosyl cellulose	I. P. C.	D.S. = 1.18 to 2.01	0	0—+
Iodotosyl cellulose	I. P. C.	D.S. = (0.60 to 0.86 iodotosyl (0.58 to 1.15 tosyl	2	0—+
Carboxymethyl cellulose	S. R. R. L.	D.S. = 0.067	56	++
Carboxymethyl cellulose, sodium salt	S. R. R. L.	D.S. = 0.2	>66	++
Carboxymethyl cellulose, aluminum salt	S. R. R. L.	D.S. = 0.067	>56	+

* E.K. = Eastman Kodak. S.R.R.L. = U.S.D.A. Southern Regional Research Laboratory. I.P.C. = Institute of Paper Chemistry. The authors are grateful to these sources for the compounds listed.

† Each value based on three or more flasks.

Experimental Methods and Results

1. Growth of Fungi on Sugar Derivatives

Qualitative observations were made on the growth of 9 species of fungi on sugar derivatives. Ten mg. of each of the compounds listed in Table I were placed in 50-ml. Erlenmeyer flasks and autoclaved at 120°C for 20 minutes. To the separate flasks there was added 1 ml. of a spore suspension from each of the various organisms. Two replicate cultures were incubated at 30°C for 3 days and then examined for mycelial growth. The results are given in Table I, with "+" indicating definite growth, "−" no growth, and "?" little or questionable growth.

2. Growth of Myrothecium verrucaria *on Cellulose Derivatives*

Studies were carried out on the susceptibility of various preparations of cellulose derivatives to attack by the active cellulose-degrading fungus, *Myrothecium verrucaria*. The shaker-flask method [5, 6] was used. One hundred mg. of the sample was placed with 20 ml. of a mineral salts solution in a 125-ml. Erlenmeyer flask. The salt solution was prepared with the following concentrations: $MgSO_4$ 0.009*M*, KH_2PO_4 0.02*M*, K_2HPO_4 0.012*M*, and NH_4NO_3 0.0375*M*. The final mixture of solution and cellulose derivatives was adjusted to pH 6.5 by 0.1*N* KOH. After sterilization by autoclaving, each flask, after cooling, was inoculated with 1 ml. of a pregerminated spore suspension and incubated on the shaker at 30°C for 9–10 days. The loss in dry weight of the substrate and the visual growth ratings were taken as indices of susceptibility. The data are given in Table II.

3. Effect of Degree of Polymerization and Degree of Substitution

Similar tests were carried out with cyanoethylcellulose preparations of various degrees of substitution. These were prepared by the reaction of acrylonitrile on cotton. Data on the susceptibility of the different samples are presented in Table III.

TABLE III. UTILIZATION OF CYANOETHYLCELLULOSE* PREPARATIONS BY *Myrothecium verrucaria*

Sample No.	% N	No. of substituents per anhydroglucose unit	% weight loss in 13 days
1	0	0	80
2	6.64	1.02	29
3	8.43	1.43	6
4	9.98	1.85	2
5	10.50	2.02	4
6	11.44	2.35	0
7	12.87	2.9	0

* Gratitude is expressed to Rohm and Haas Co. of Philadelphia for this series of preparations.

In the next series 4 species of fungi and one of bacteria (*Sporocytophaga myxococcoides* USDA 482), which had been isolated from deteriorating cotton, were used. Cellulose acetates with varying degrees of substitution and polymerization were employed as sole sources of carbon for the organisms. The acetates were prepared by Dr. E. Heuser of the Institute of Paper Chemistry by the acetylation of cellulose in phosphoric acid solutions [4]. The acetate with a low degree of substitution and a low degree of polymerization was prepared by acetylating dissolved cotton linters in 100-percent phosphoric acid with a limited quantity of acetic anhydride without cooling for about 1½ hours, followed by precipitation with diethyl ether. By means of different combinations of temperature and duration of acetylation, he succeeded in obtaining highly or lowly substituted acetates with either a high or a low degree of polymerization. Inasmuch as the reaction occurred in solution, it is assumed that partial acetylation resulted in a more or less uniform distribution of acetyl groups along the anhydroglucose chain.

Four samples of acetates prepared in this way were subjected to microbiological attack in shaker

TABLE IV. DEGRADATION OF CELLULOSE ACETATES BY MICROORGANISMS

Sample No.	Description	% acetyl	Degree of polymerization	*M. verrucaria*	*A. flavipes*	*G. convoluta*	*C. lunata*	*S. myxococcoides*
1	High acetyl, high D.P.	37.9	510	0.0	0.0	0.0	0.0	0.0
2	High acetyl, low D.P.	40.0	100	0.0	0.0	0.0	0.0	0.0
3	Low acetyl, high D.P.	4.8	310	68.5	41.0	37.6	18.6	48.4
4	Low acetyl, low D.P.	3.2	150	64.9	34.2	39.7	13.4	52.8

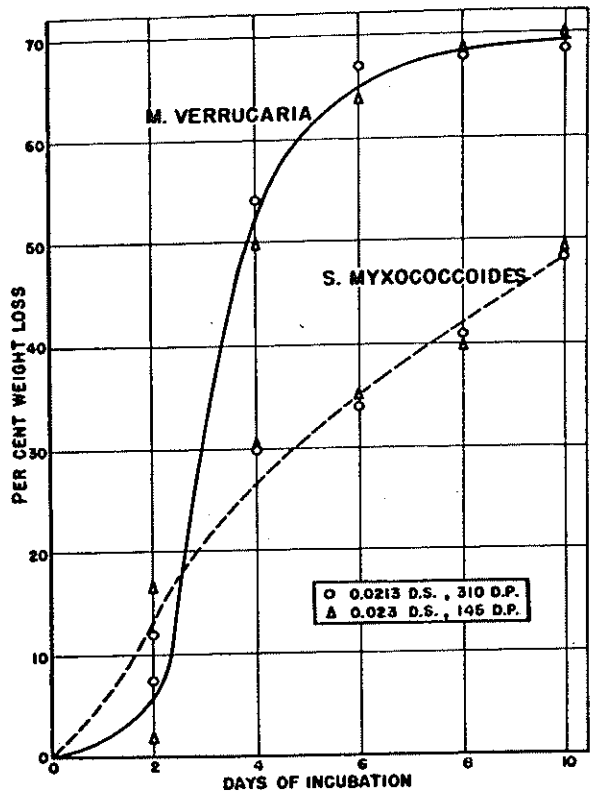

FIG. 1. *Growth of microorganisms on cellulose acetate.*

flasks. The susceptibility of the preparations to these microorganisms during a period of 10 days at 30°C is given in Table IV.

Results of experiments on rate of growth were conducted with *S. myxococcoides* and *M. verrucaria* and are plotted in Figure 1. Similar experiments with triacetates varying in D. P. from 61 to 510 all showed complete resistance to microorganisms.

Discussion

The results given in Table I reveal the high specificity of microorganisms in the utilization of sugar derivatives. Whereas the unsubstituted glucose, mannose, cellobiose, and the glucoside supported growth, any substitution on the pyranose ring resulted in increased resistance to microbiological attack. Apparently neither the nature nor the number of substituents per ring determines the extent of resistance. Any modification of the ring prevents the microorganisms which have been studied from attacking the substrate. The same situation seems to obtain in the case of derivatives of cellulose, as revealed by the data in Table II.

At the same time, however, data in Table II suggest that complete resistance is exhibited only by those preparations with a degree of substitution above one. This indication is further supported by the measurements recorded in Table III. In these experiments it may be seen that samples with 1.43 or more substituents per anhydroglucose unit are all completely resistant to attack by *Myrothecium verrucaria*. Although pure cellulose was digested by the organism to the extent of 80 percent in 13 days, only 29 percent of a cyanoethylcellulose sample (average of 1.02 substituents per anhydroglucose unit) was similarly degraded in the same length of time. Inasmuch as the cyanoethylation was carried out on native fibrous cellulose, it is likely that the sample was not uniform—*i.e.*, it is probable that the loss in weight of 29 percent reflects the behavior of the unreacted fraction of the cellulose in the sample, or, as will be pointed out in the subsequent paragraph, the unsubstituted anhydroglucose units.

Further insight into the mechanism of breakdown of the cellulose molecule may be inferred by examination of the data given in Table IV and Figure 1. The degree of substitution in samples Nos. 3 and 4 represents approximately one substituent of every four anhydroglucose units. Because of the method used, which involves homogeneous acetylation of cellulose in solution, it is reasonable to assume that these substitutions are uniformly distributed along the chain. Total utilization of all the unsubstituted anhydroglucose units in these derivatives should yield a value of approximately 74 percent of their loss in weight. As shown in Figure 1, the actual experimental value approximates 70 percent. The rather close agreement strongly suggests that the organism can attack a glycosidic linkage or an unsubstituted anhydroglucose unit at any position in the cellulose linear chain. It need not *initiate* the attack at the ends, and our interpretation is contrary to Clayson's belief [1] that the cellulolytic action on the molecule proceeds end-wise.

An interesting speculation can be made as to whether the end or the internal linkages are the more susceptible to degradation. If it is assumed that acetylation occurs at random throughout the anhydroglucose units in the cellulose chains (samples Nos. 3 and 4), it then follows that with the same degree of acetylation a sample with a smaller chain length will have a greater proportion of un-

substituted anhydroglucose units at the ends of the chains. The fact that equal rates of utilization of the two samples occur seems to imply that free anhydroglucose units are equally susceptible to attack by microorganisms regardless of their position in the substituted chain. Unfortunately, the differences in chain length between the two substrata are too small and the degree of acetylation is too high to provide a critical evaluation of this hypothesis.

The importance of these theoretical considerations on the potentiality of "multipurpose" topochemical reactions on cotton fibers is highly significant. It appears that in order to impart mildew-resistance to cotton fibers it is necessary only that all anhydroglucose units of the cellulose molecules in the *surface fiber* layers be substituted. Furthermore, it is evident that only one substituent per anhydroglucose unit is necessary. Fortunately, as has been shown, the nature of the substituents may be varied within wide limits. Considerable promise exists, therefore, that from the wide array of possible reactants a number may be selected on the basis of ability to impart additional desirable properties to the topochemically modified cellulosic fabric.

Summary

Experiments on the growth of various selected species of fungi and bacteria isolated from deteriorated cotton textiles uniformly reveal their inability to utilize derivatives of glucose, mannose, cellobiose, and cellulose. Despite a wide range of substituents on various carbohydrates, almost complete, if not complete, resistance to microbiological attack was exhibited by the compounds tested.

In the case of cellulose, the available data strongly suggest that microorganisms are capable of digesting unsubstituted anhydroglucose units at any position in the chain of a partially substituted cellulose derivative.

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